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Evaluation of Separation and Purification Processes in the Antibiotic Industry

Scientific Note

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Index Entries: Antibiotics; separation and purification; supercritical fluid extraction; chromatography.

INTRODUCTION

Antibiotics are a class of antimicrobial chemicals that are produced by microorganisms (mainly the saprophytic molds and bacteria of the soil). These substances will inhibit the multiplication of various microorganisms or may destroy these organisms by interfering with cell wall development and/or metabolism. Each antibiotic has its own characteristically selective "spectrum" of potency against various microorganisms, which varies not only for the species, but also for the strain of the infecting organism and other conditions. The term "broad spectrum" is applied to antibiotics that are effective against a wide range of bacteria (*1*). In general, antibiotics are ineffective against typical viral diseases. With the significant exception of the penicillins and cephalosporins, which are produced by molds, all major classes of antibiotics are derived from bacteria (*2*).

The three most important classes of antibiotics, from both a commercial and clinical standpoint (Table 1 (*3*)) are the penicillins, cephalosporins, and tetracyclines. Over 100 different penicillins have been produced by natural fermentation, however only penicillin G, penicillin V, and to a

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Table 1
US Antibiotic Production (1985)

Antibiotic	Production, $ Millions
Cephalosporins	1211
Penicillins	252
Tetracyclines	280
All others	754
Total:	2497

lesser extent penicillin O are produced in commercial quantities. Table 2 (*4,5*) gives some of the important physical properties of the penicillins. All three penicillins are produced by the same process, with the constitution of the growth media determining the specific penicillin that is produced. All of the penicillins are available in crystalline form, being stable for several years under controlled conditions. Penicillin V is normally administered orally, while penicillin G is mainly administered in solution form, where its stability is rather limited. Over 20 therapeutically useful semisynthetic penicillins are derived from penicillin G by biosynthesis.

The penicillins are produced by submerged culture fermentation in 40,000–200,000 L fermenters. While many organisms can be used to produce penicillins, the yield is strongly dependent on the strain that is used. In general, the concentrations of antibiotics are very low, however with proper strain selection penicillin can be produced in concentrations up to 40 g/L, which is one or two orders of magnitude greater than many other antibiotics.

Table 2
Summary of Physical Properties

	Molecular weight	Melting point °C	Solubility[a] water	Solubility[a] ethyl acetate	Solubility[a] ethanol
Penicillin V	350.0	124.0	9.0	>20.0	>20.0
K salt	388.0	263.0	>20.0	0.65	1.34
Penicillin G	334.0				
Na salt	356.0	215.0	>20.0	0.40	9.97
K salt	372.0	215.5	>20.0	0.42	10.4
Tetracycline	444.0	172.5			
Cephalosporin C	415.4				
Cephalothin	382.4	160.5			
Na salt	404.4		>20.0	0.02	17.7
Cephaloridine	399.0		>20.0	0.035	1.95

[a]Solubility in g/L at 294–301°C.

The cephalosporins are economically and therapeutically as important as the penicillins. These drugs are broad-spectrum antibiotics that have low toxicity. There are six natural and 13 semisynthetic cephalosporins that are therapeutically important. They are produced by the same process used for the penicillins, utilizing a different growth medium and organisms; however the final fermentation broth concentrations are one to two orders of magnitude lower, resulting in a more difficult separation and purification process.

Tetracyclines are an important class of broad-spectrum antibiotics active against both gram-positive and gram-negative bacteria. There are six natural and six semisynthetic antibiotics of therapeutic importance. Like the penicillins and cephalosporins, the tetracyclines are produced by a similar fermentation process except that the organism is a bacteria instead of a fungi. Presently there are about 20 different organisms which can be used to produce tetracyclines. The current industrial yield is low. The patent literature (*6,7,8*) suggests concentrations of 0.2 to 2.0 g/L.

Antibiotic manufacturing plants contain two main processing segments; a highly specialized fermentation section and a large, energy-intensive separation and purification section. While the fermentation section accounts for only a relatively small portion of the total facility Fig. 1 (*9*), highly specialized equipment (fermenters, heat exchangers, pumps, and so on) and operating conditions (large volumes of sterile air, a sterile environment, growth media, organisms, low temperature cooling water, and so on) are required.

The separation and purification section employs standard equipment for the most part. However, this section is large and sterile conditions are required. The separation of the antibiotic from the fermentation broth and subsequent purification steps can require up to 60 separate unit operations. Liquid extraction is employed extensively using various organics such as amyl acetate, butanol, butyl acetate and so on. These solvents in turn must be purified by distillation and recycled, an energy-intensive process. A first step toward rebuilding the antibiotics industry after a national disaster would require a documentation of the many different separation and purification methods that are currently in use.

CONVENTIONAL SEPARATION TECHNOLOGIES

All antibiotic fermentations use similar equipment, while different antibiotics are produced by using different cultures and growth media. Some alterations in operating conditions may be required for optimum performance. The separation and purification sections of an antibiotic plant can differ substantially depending on the specific antibiotic that is being produced and enduse purity requirements. Filtration, centrifuga-

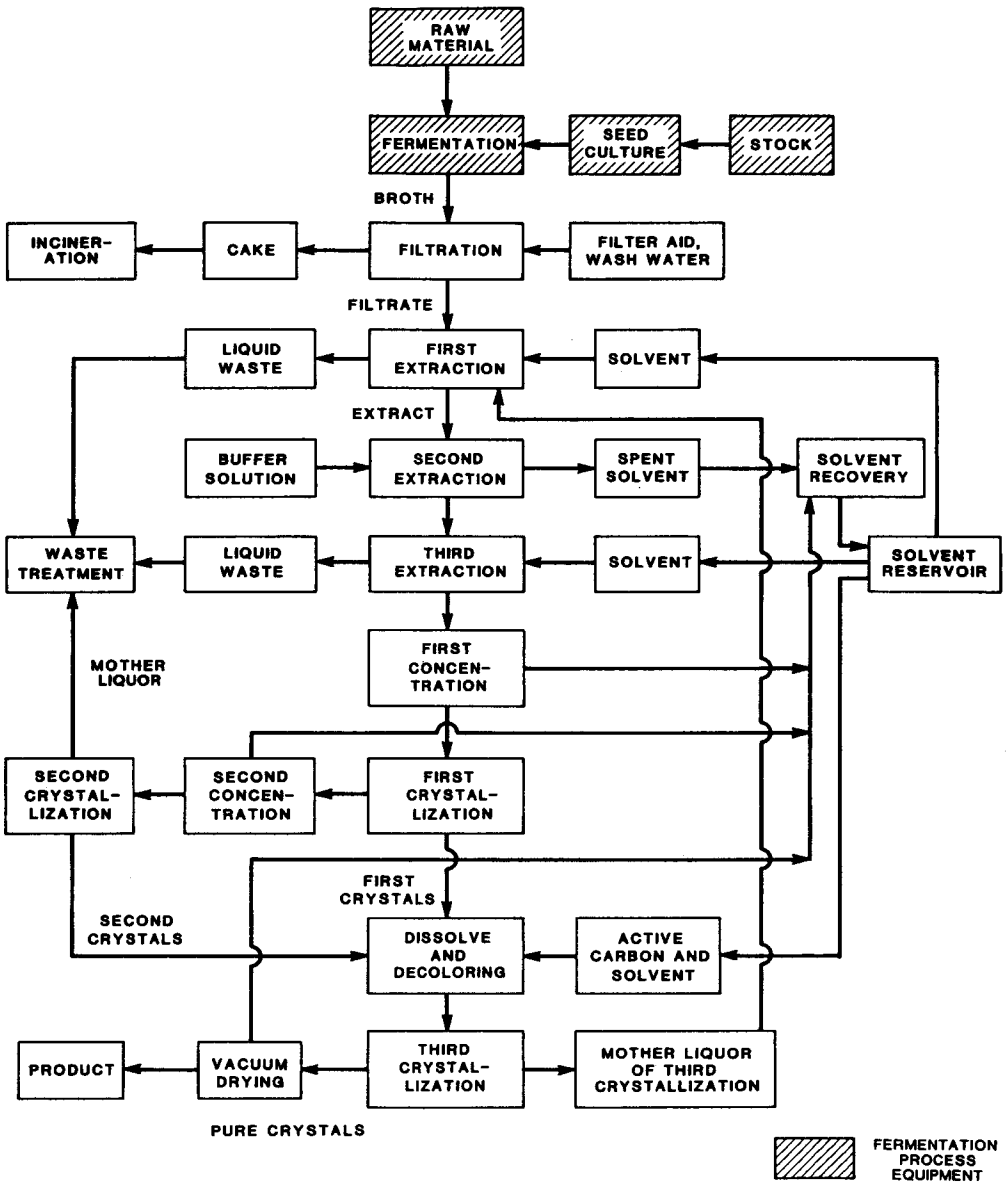

Fig. 1. Block diagram for a general antibiotic production process.

tion, liquid–liquid extraction, and crystallization are generally employed. Usually several different separation methods are required in a repetitive manner for a given antibiotic.

The separation and purification must be conducted under sterile conditions to avoid denaturing the drug or restricting the conditions under which it can be used. Many antibiotics are subject to rapid degradation in the soluble form, placing time restrictions on the separation and

purification processes. In general, antibiotics are sensitive to high temperatures, eliminating any type of separation process that operates at temperatures well above ambient.

The many different separation and purification methods currently in use for the three major families of antibiotics will be documented. The most common separation method currently in use will be described in some detail for each of the antibiotic families.

Penicillin

Many different processes have been used commercially to recover penicillin from the fermentation broth (*2,5,10–13*). The general flowsheet

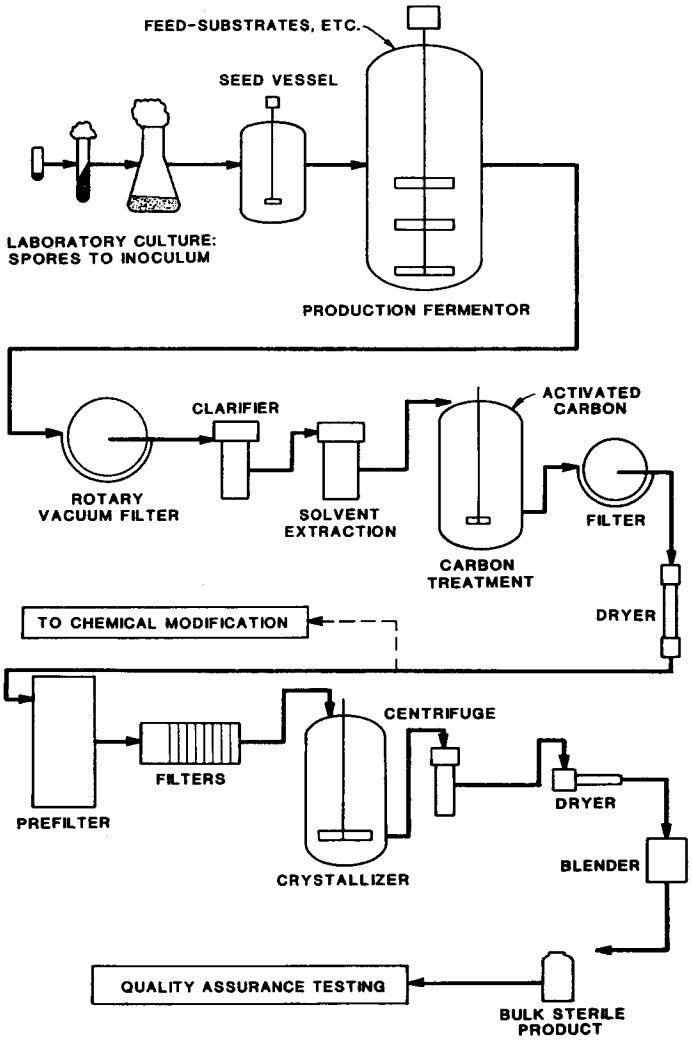

Fig. 2. General production scheme for penicillin.

is given by Fig. 2 (*31*). The process takes about 15 h. At the end of the fermentation, the penicillin is in an extracellular solution with other metabolites, the growth media, and cells. The first step is the removal of the cells via filtration under conditions that will prevent contamination of the filtrate with β-lactamase producing organisms.

The penicillin rich filtrate is now cooled to 0–4°C to minimize degradation. The drug is isolated from the solution, usually by solvent extraction. In an aqueous solution at a pH of 2.0–2.5, there is a high partition coefficient in favor of certain organic solvents such as butyl acetate, methyl isobutyl ketone, and amyl acetate. The penicillin-rich extract is treated with 0.25–0.5% activated carbon to remove pigments and other impurities. The purified penicillin can then be extracted back into an aqueous buffer at a pH of 7.5. The drug is recovered from solution by adding sodium or potassium acetate and precipitating it as a metal salt. The crystals are washed and predried with anhydrous *i*-propanol, *n*-butanol or other volatile solvents, with final drying being accomplished using a vacuum or warm air (*10,11*).

Cephalosporin

The cephalosporins are structurally very similar to the penicillins, and several are produced from penicillin by biosynthetic means. The cephalosporins that are produced from fungi are extracellular fermentation products, which allows bulk filtration to be used to separate the solid matter from the antibiotic containing broth. Recovery from the filtrate is difficult because of the low product concentration and the need to remove high molecular weight (1000 mw) biological compounds that can lead to allergic and toxic reactions when the drugs are administered. Final broth concentrations range from 1.0–4.0 g/L for cephalosporin C (*14*).

Many different separation and purification schemes are employed, including conventional solvent extraction, ion exchange resins, and salting out procedures. The general procedure is to filter the fermentation broth at acidic pH (5.0), followed by adsorption of the filtrate on activated carbon, removal of the adsorbed antibiotic by contacting the carbon with a mixture of water and a polar organic solvent, contacting the eluate with an anion exchange resin, and eluting the resin with a salt solution at a pH of 5.5–10.0. Frequently, the carbon adsorption steps are replaced with a precipitation step. Many different precipitations are possible. Among the more common are (*15*)

1. Crystallization of the potassium or sodium salt from purified aqueous solution of the cephalosporin by concentration and/or addition of large volumes of a miscible solvent.
2. The zinc salt (also copper, nickel, lead, cadmium, cobalt, iron, and manganese) can be crystallized from purified aqueous solutions.

3. Insoluble derivatives such as the *n*-2,4-dichlorobenzoyl cephalosporin and tetrabromocarboxybenzoyl cephalosporin are crystallized as the acid from solution.
4. Sodium-2-ethyl hexanoate will precipitate the sodium salt of *N*-derivatized cephalosporins from solvents.

Tetracycline

Tetracycline antibiotics are produced from bacteria of the soil. A general flowchart for the separation and purification of tetracyclines is given by Fig. 3 (*16*). Isolation methods for the tetracyclines must take into account their amphoteric nature and the possibility of their polymerization or rearrangement. Several of the more common methods for recovery and purification of tetracyclines are outlined below (*7*).

1. Adsorption on diatomaceous earth or activated charcoal with subsequent chromatography or selective extraction.
2. Extraction from acid or alkaline medium. The most frequently used extraction agent is 1-butanol, owing to its suitable partition coefficient and economic availability.
3. Direct mash extraction based on solubilizing the antibiotic by acidification, precipitation of Ca^{+2} with ammonium oxalate, addition of quaternary ammonium compounds as carriers, and extraction of the metabolite with an organic solvent, usually one of the methylalkyl ketone type.

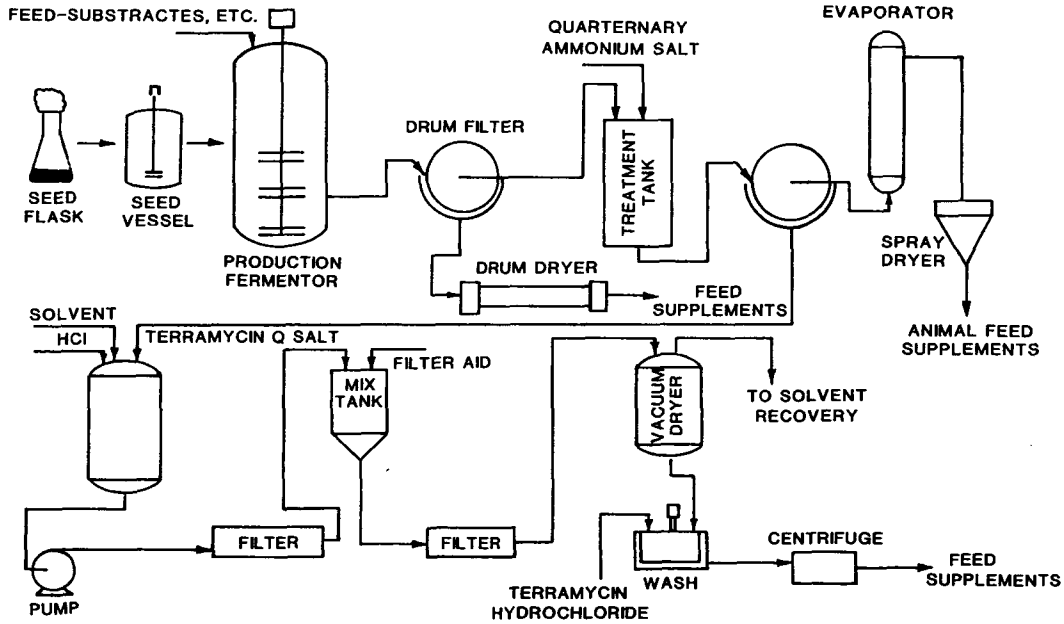

Fig. 3. General production scheme for tetracyclines.

4. Precipitation (dry salt) process based on precipitation of the antibiotic from dilute aqueous solution of aryl azosulfonic acid dyes. Tetracyclines are precipitated as complexes with alkaline earth metal compounds or with primary and secondary alkyl amines.
5. Solvent extraction of the antibiotic with salt, based on salting out (NaCl) the antibiotic from the aqueous to the organic phase (1-butanol). This method is also suited for refining a crude product.

One tetracycline, oxytetracycline, is purified by combining the release of oxytetracycline into the medium by acidification, precipitation of ballast compound with $K_2Fe(CN)_6$ and $ZnSO_4$, extraction of the liquid fractions with butyl acetate, and precipitation of Ca^{+2} with oxalic acid. After adjusting the filtrate with EDTA, Na_2SO_3, and citric acid, the crystalline base of oxytetracycline is obtained.

Further purification is carried out by crystallization as salts (e.g., hydrochlorides) or bases. Particularly efficient is crystallization from boiling solvents, such as lower alcohols, ketones, or aliphatic ethers of ethylene glycol, which yield nonhygroscopic preparations. Residual amounts of antibiotic in the mother liquor are increased by oxalate and chloride ions, while sulfate anions have the opposite effect. Crystallization is most efficiently performed at 2°C for 3 h (*17,18*).

ADVANCED SEPARATION TECHNOLOGIES

Three relatively new and potentially very powerful separation/purification methods for antibiotics are chromatography, supercritical fluid (SCF) extraction, and membranes. If the size of the antibiotic manufacturing process is to be substantially reduced, this reduction will have to come in the separation and purification sections of the plant, which represents 60–80% of a typical plant. Chromatography, SCF extraction, and membrane technologies are relatively new in regard to their application to the antibiotic process. Most of this work is still in the developmental stages, although some penicillins and cephalosporins are separated commercially using HPLC and/or membranes (*19,20*).

Chromatography

Chromatography has been known for many years and used very effectively as an analytic tool. Recently there has been a great deal of interest in using preparative chromatography as a commercial separation and purification device for antibiotics (*19–31*). This research centers around the development of highly selective resins, and scaleup procedures including proper operating parameters. The major problems with a prepar-

ative chromatographic separation process are the adverse effects of increased column diameter on the resins and flow properties in the bed, and the batch nature of the process.

One way of making a continuous chromatographic process would be to use the annular chromatograph developed by Scott et al. (*32*), depicted in Fig. 4. The annular chromatograph is not yet in use commercially, however its large scale use has been demonstrated and the design equations developed (*29,33*).

Several patents (*30,31*) exist in the literature for the recovery of cephalosporin antibiotics using HPLC. Fig. 5 (*20*) gives a potential process flowsheet. The HPLC columns depicted are 60 cm in length and 20 cm in diameter. A special problem encountered in the manufacture of many cephalosporins is the contamination of the drug with relatively high weight proteinaceous materials and peptides from the fermentation broth. These contaminates cause allergic and toxic manifestations upon administration (*30*). HPLC has been found to be an effective method of removing these contaminates when used as a final purification step.

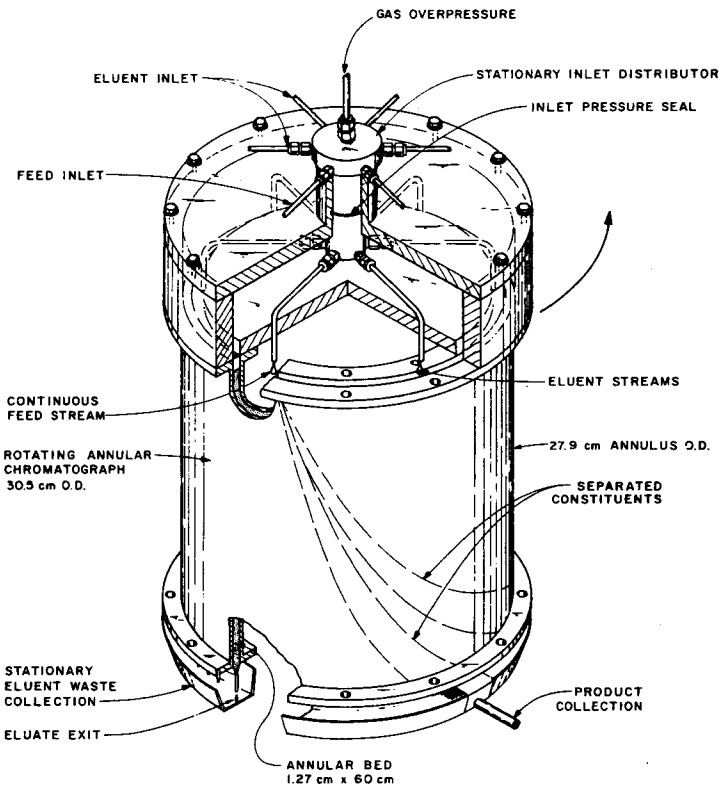

Fig. 4. Detailed diagram of an annular chromatograph.

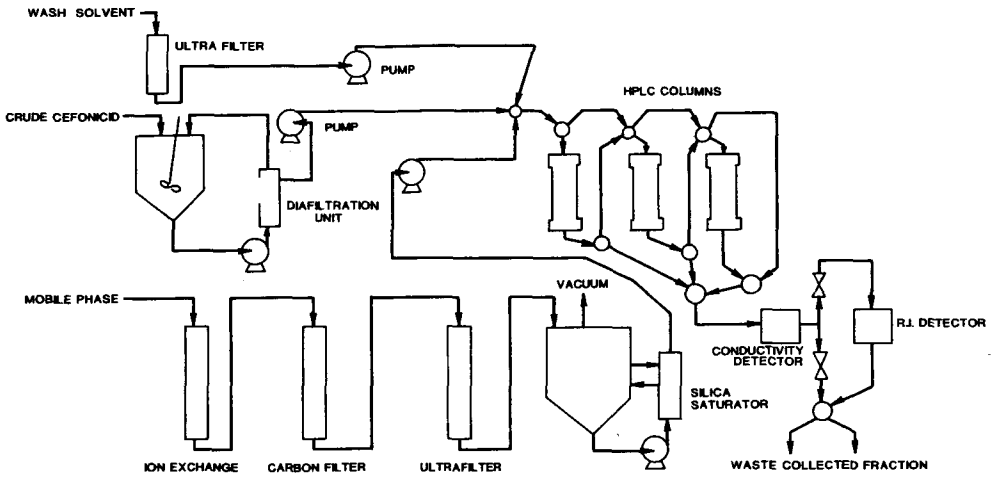

Fig. 5. Flowsheet depicting antibiotic separation and purification using chromatography.

Supercritical Fluid (SCF) Extraction

The solubility of solids in SCF was discovered over 100 years ago. Supercritical extraction is similar to conventional liquid extraction except in this case a compressed gas, rather than a liquid, is contacted with a solid or liquid mixture in order to selectively extract one or more components. In the critical region (reduced temperatures of 1.0–1.2), fluids exhibit large changes in density and dielectric constant with changes in pressure and temperature. The density of an SCF can approach that of the condensed phase. Since the solubility of a nonvolatile substance is strongly dependent on the solvent density and dielectric constant, SCF solvents can be used to extract heavy (low vapor pressure, high molecular weight) substances.

A unique property of this process is that the dissolved substance may be separated by decreasing the fluid density. This is accomplished by expanding the mixture by either decreasing the pressure or increasing the temperature. The key point is that a small change in temperature or pressure will result in a large change in solvent density and thus a large change in solubility. Recently SCF extraction was applied commercially to the decaffeination of coffee and has generated widespread interest in the pharmaceutical industry (*29,34*) because this separation technique can be performed at low temperatures (20–60°C). Figure 6 presents a schematic diagram of the basic process.

Conventional liquid–liquid extraction, which is quite common in the antibiotic industry, often requires a difficult crystallization, a distillation and/or use of a second solvent. A solvent residue may be left on the product. With SCF extraction the extract is separated by reducing the solvent

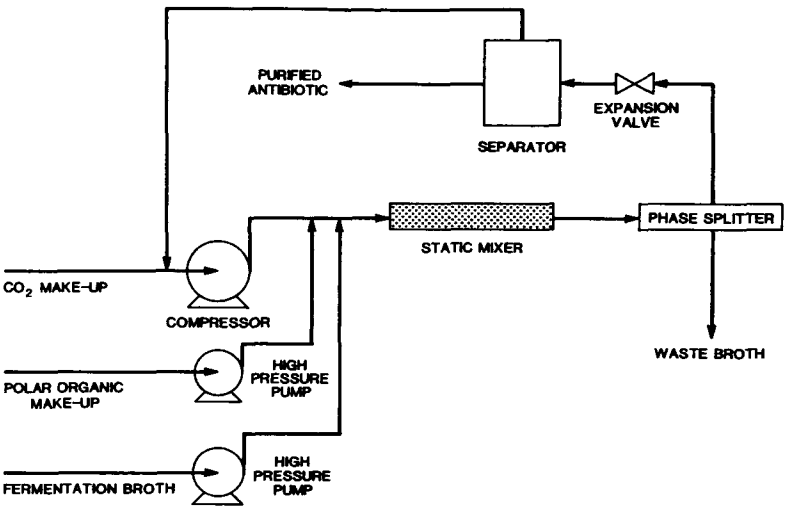

Fig. 6. Schematic diagram for separation/purification of antibiotics using supercritical fluid extraction.

density to the gaseous state, thereby leaving very little solvent on the product. In addition, the transport properties of SCFs are closer to those of the gaseous state than the liquid state. These enhanced diffusivities and viscosities will minimize any mass transfer problems in the extraction. Recently a patent (*35*) was issued for the separation and purification of antibiotics using supercritical fluids. The major advantages would be a possible reduction in the number of processing steps and elimination of the large and expensive solvent recycle system.

Many different SCF solvents are possible as extracting agents. Table 2 shows that the solubility of penicillin V in ethanol is over twice what it is in water (the major constituent of the fermentation broth). In the supercritical region, ethylene and water are immiscible with ethanol distributing between the two phases. It is reasonable to postulate that if a SCF mixture of ethylene and ethanol were used for fermentation broth extraction, penicillin V would likely be concentrated in the SCF phase.

Membranes

Membranes are defined as imperfect physical barriers between two fluid phases. Transport of dissolved substances between the two fluid phases is determined by the physical characteristics of the membrane and properties of the dissolved substances. Two important properties of a membrane are the permeability, the rate at which a given component is transferred through the membrane under defined conditions, and the permselectivity, the ratio of the permeation fluxes of two components through the membrane under identical driving forces (*2*). Membranes

can separate either by molecular size or chemical properties such as electron charge, and so on.

Membranes are attractive for antibiotic separation and purification because they operate at ambient temperature, can be very selective are easily scaled up, and will operate with high flow rates (*29*). Figure 7 (*19*) illustrates the basic concept. Recently membranes have been used for the isolation of cephalosporin C from the fermentation broth (*19,27*).

CONCLUSIONS

Antibiotic production is a complex capital intensive process, which divides naturally into two segments, fermentation and separation/purification. The separation and purification section is very large as a result of the number of processing steps required (up to 60) and the need to purify and recycle large quantities of organic solvents. Separation and purification is not generic within the antibiotic industry. Not only does each individual antibiotic require a different separation process, but also there are many different separation schemes in use for the same antibiotic. Much research is currently in progress on three relatively new separation techniques on a commercial level, which may lead to substantial reductions in the complexity of the process; chromatography (both conventional preparative HPLC and annular chromatography), supercritical extraction, and various membrane processes.

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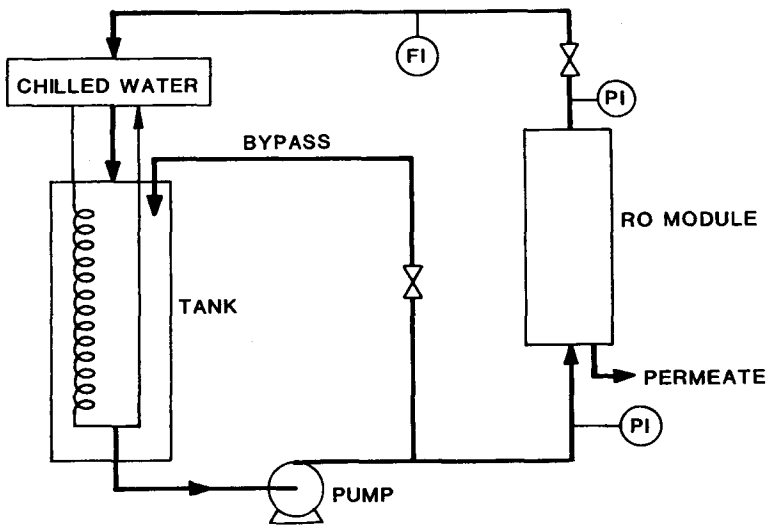

Fig. 7. Reverse osmosis concentration of cephalosporin C.

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